Light-programmable Logic Gate

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Abstract

This paper describes a new method of retroactively programming a genetic circuit to exhibit the behavior of one of four logic gates (AND, OR, NAND, and NOR) using pulses of light. These pulses must occur after DNA transfection into the host cell. The pulses of light deactivate a photosensitive kinase, inhibiting phosphorylation. Using this effect, we can control the transcription of recombinase enzymes that can "reprogram" our DNA-based circuits through excision of particular sections of DNA, as determined through placement of recombination sites. We also describe a novel circuit design for an "undifferentiated logic gate" that can be reduced to any of the four basic logic gates through the introduction of reecombinase to the system.

1 Introduction

Right now, genetic circuits are designed to exhibit some static behavior that does not change once the DNA has been delivered to a cell. A transcriptional AND gate will remain so for the lifetime of the DNA. This is a bottleneck to the acceleration of abstraction and modularity in the field of synthetic biology. Complex components have to be assembled using carefully engineered processes like Gibson assembly, even to implement a relatively simple logical behavior.

However, there are some ways of changing a circuit post-assembly and post-transfection that have begun to be explored. One of these is recombinase. This enzyme has the ability to excise or reorient strands of DNA, depending on the orientation of its unique recombination sites on the strand. By using a simple, ubiquitous input like light to retroactively determine the behavior of genetic circuits, we enable a new level of modularity to synthetic biology.

2 Controlling transcription with light

Central to this mission is a method of modulating transcription with light. A method for doing this has already been designed and engineered successfully by Tabor et. al. [2] in the creation of their edge detection circuit.

Their circuit utilized a chimeric transmembrane enzyme with a photosensitive domain (presented on the cell surface) and an internal kinase domain.[2] The ability of the kinase to phosphorylate a response regulator protein is impeded

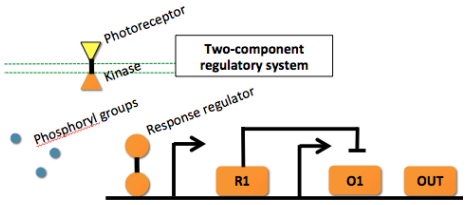


Figure 1: This circuit turns on the transcription of the OUT protein in the presence of light.

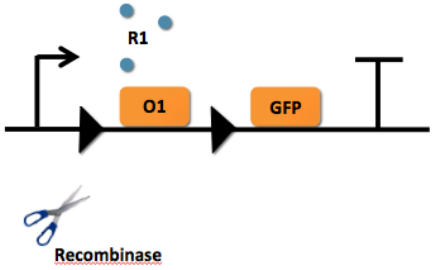


Figure 2: The introduction of recombinase (here represented by scissors) to this circuit would lead to the excision of the operator sequence. This would obviate the presence of the repressor molecules (represented by blue dots). If the two recombination sites instead were inverted with respect to each other (the black triangles pointed towards O1), then the recombination would have "flipped" the operator upside down instead of excising it.

by the presence of light shining on the photosensitive domain. Thus, when light is shining on the cell, the response regulator is not properly phosphorylated, and transcription is repressed. If the cell is in darkness, the response regulator of completely phosphorylated and transcription continues. By inverting the output of this light-sensitive circuit, we have a circuit that transcribes a protein in the presence of light.[1]

By using photosensitive domains that are sensitive to different frequencies of light, we can control the transcription of many different proteins entirely using light.

3 Using recombinases to modify genetic circuits

Recombinase is an enzyme that can modify DNA by excising portions or reversing the orientation of a given segment. [3]

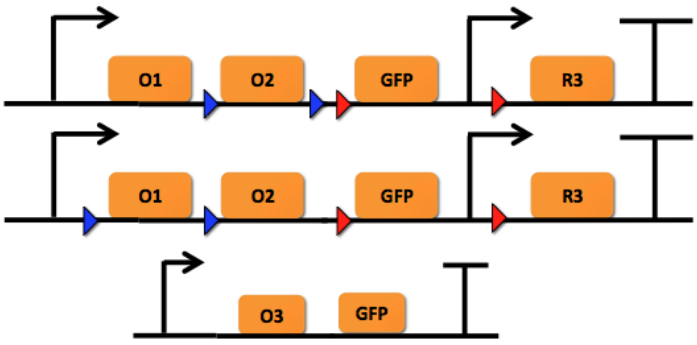


Figure 3: The "undifferentiated" logic gate.

4 Circuit design

The key innovation contained herein is the novel circuit design of an "undifferentiated" logic gate that can be assigned as an AND, OR, NAND, or NOR gate using merely pulses of light. The resulting gates are transcription based and have inducers as inputs. It is assumed that R1 and R2 are being produced constitutively or provided to the cell exogenously. The output of the gates is GFP.

The two pulses of light provide two bits of information as inputs, which map to the four possible states of the logic gate. A diagram of this circuit is below. There are three distinct DNA strands interacting, which I will refer to as the "top", "middle", and "bottom" strands.

Based on the introduction of the recombinases, whose color-coded binding sites are shown as pointing triangles, this circuit reduces to one of four transcription-based logic gates whose diagrams are also shown in the table for reference.

If blue light is shined on the cell, the blue recombinase is transcribed. This excises the operator O1 on all middle strands and O2 on all top strands. Thus the circuit has reduced to an OR gate – the presence of either inducer 1 or inducer 2 is enough to initiate transcription of GFP. The promoter for R3 is constitutive, thus permanently deactivating all bottom strands.

If red light is shined on the cell, the red recombinase is transcribed. This excises the GFP and promoter all middle strands and top strands. These strands now require both I1 and I2 in order to activated transcription of R3. Transcription of R3 would turn off transcription of GFP. Thus we have a NAND gate, where output is high unless both inputs are high.

If both colors of light are shined on the cell, both recombinases are transcribed. This excises the GFP and promoter all middle and top strands, O1 on middle strands, and O2 on top strands. We now have a circuit where either inducer will initiate transcription of R3, thus turning off the output. Thus we

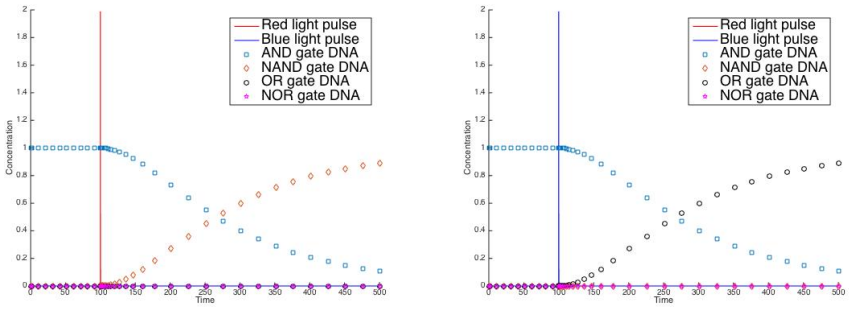


Figure 4: This shows the concentrations of each logic gate type in the time following a single light pulse, red on the left and blue on the right. Note that the undifferentiated DNA acts as an AND gate, which is why that form is dominant initially.

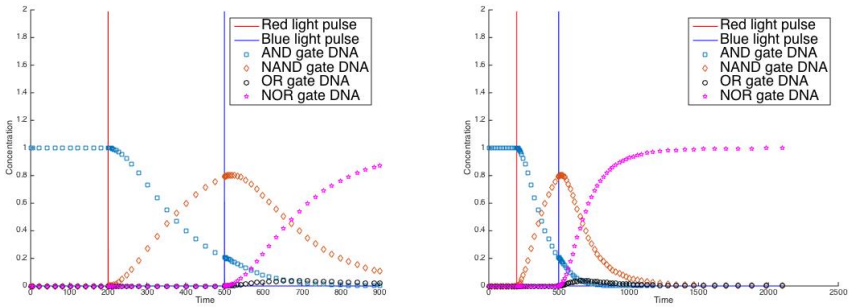


Figure 5: This shows the concentrations of each logic gate type following pulses of both color, spaced apart somewhat. Notice the pulse of NAND DNA following the red pulse, which begins being reduced to NOR by the integrase expressed following the blue pulse.

have a NOR gate: the output is only high if both inputs are low.

5 Modeling and Results

I modeled this system using discretized differential analysis in MATLAB.

This behavior is exactly what we expected. The modeling was fairly simplistic, involving a simple model of transcription involving only DNA -¿ mRNA -¿ protein reactions. Both proteins and mRNA have a set degradation rate. The integrase-DNA reaction occurs very fast and has no reverse reaction.

Future directions for this work involve using photomasks to give cells in different regions dramatically different "firmware." Generalizing, it would be possible to build a synthetic light programmable gate array (LPGA) which has the possiblility to dramatically increase the complexity of combinational logic

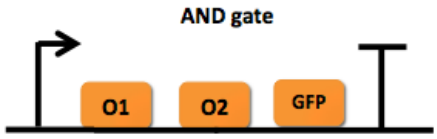
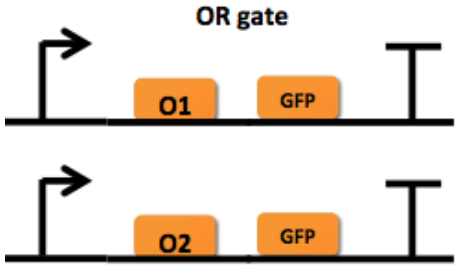
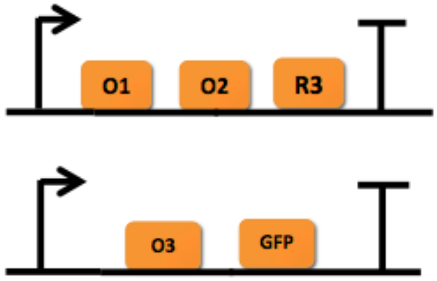
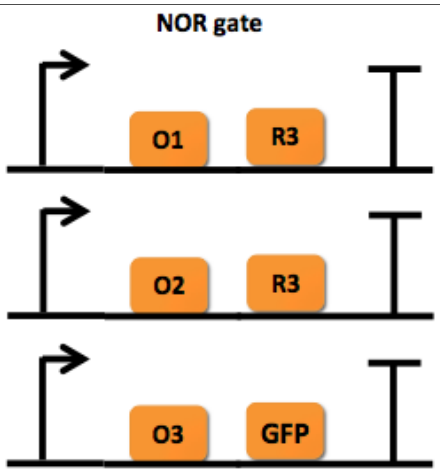
Recombinase	Logic Gate
None	
	
	
	

Table 1: Mapping each combination of recombinases to the resulting logic gate.

possible to implement synthetically.

Most interesting about this project is the trend in represents towards "assembly line" manufacturing of complex biological devices. The same genetic circuit can be manufatured at huge scale, then programmed post-transfection to display a desired behavior, even using an input as simple and ubiquitous as light.

References

[1] Alexander Y. Mitrophanov and Eduardo A. Groisman1 *Signal integration in bacterial two-component regulatory systems* Genes Dev. Oct 1, 2008; 22(19): 2601–2611. 2008.

[2] Tabor JJ, Salis HM, Simpson ZB, Chevalier AA, Levskaya A, Marcotte EM, Voigt CA, Ellington AD. *A Synthetic Edge Detection Program.* Cell, 137 (2009), pp. 1272–1281 2009.

[3] Piro Siuti, John Yazbek, and Timothy K Lu Synthetic circuits integrating logic and memory in living cells Nature Biotechnology 31, 448–452 (2013).